Evaluation of the applicability of regression equations for sorting commingled remains on 3-Dimensional bony elements from CT scans

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KEYWORDS: Forensic science; Forensic anthropology; CT scans; 3-D Segmentation and reconstruction; Human Identification; Commingled scenarios.

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Highlights (for review)

- The quality of the models' surface affects the efficacy of the regression models
- Denoted a lack of clear statistical procedure in the regression models
- Further research needed on the quality of the scans and segmentation procedure

Abstract: This pilot study examines the applicability of osteometric models for addressing commingled remains, which were originally developed for dry specimens, on 3-Dimensional bony elements in relation to a modern cadaveric population. A total of 70 bony elements (humeri, radii, ulnae, femora, tibiae and fibulae) were segmented and virtually reconstructed from cadaveric whole-body CT scans. Linear measurements were taken (using MeshLab v.2016.12) of the 3-Dimensional elements and osteometric models for sorting applied to them (Byrd and Adams, 2003). This study showed that on the selected specimens the quality of the surface of the reconstructed specimens compromised the efficacy of the models, and consequently the reliability of the results.

Introduction

The identification of victims of mass fatality events, either man-made events (e.g. terroristic attacks) or environmental catastrophes (e.g. earthquakes), is a priority for the personnel involved in the investigations, as affirmed by Interpol in the RESOLUTION No. AGN/65/RES/13 (1996). Computed Tomography (CT) scans are among the methodologies which have become integral to this identification process (Brough et al., 2015). Additionally there is a requirement that all techniques utilised must be able to satisfy the standard of reliability and validity required to perform the examinations of remains and be acceptable in a courtroom (National Research Council 2009, The Law Commission, 2011, and Executive Office of the President, 2016).

This study aims to examine whether techniques developed for osteometric sorting of dry bones (e.g. regression equations) can give reliable results when applied to virtual models especially in relation to the reliability of osteometric sorting of commingled scenarios, when applied to these virtual bony elements.

Materials and Methods

Materials

Twelve cadaveric CT scans, six of which represented whole bodies and six of which represented partial ones, have been used for this study. They were created from already existent scans of individuals who had donated their bodies for research. The decision for utilising pre-existent materials relied on the recognition of ethical issues that exist

around the exposition of living subjects to ionising radiation or in the creation of a commingled scenario from whole human remains (Isaza et al., 2014).
A serial number was assigned to each scan and the examiner had no access to any personal information on the subjects in order to not violate the privacy and ethics around the Body Donation Program. The details in possession of the examiner were limited to the patient serial number ID, the name of the institution in which they were scanned, the date of the series, the modality (through CT scan), the series' description, the body part examined and the position in which the patient was scanned.
Each scan was uploaded and visualised in DICOM format on a workstation using the software AMIRA v.5.3.0.

For this study, it was decided to visualise and export 3-Dimensional models of the six main long bones from both sides of the body: humeri, ulnae, radii, femora, tibia and fibulae of each subject (See Table1 for additional details). These regions were chosen because it allowed the evaluation of the left and right side against each other. Moreover, the existent literature in the field reports that the six main long bones are more likely to be preserved either completely or partially in a commingled scenario (Steel and McKern, 1969, Simmons et al., 1990, Holland, 1992, Adam and Byrd, 2006, Chibba and Bidmos, 2007, Bidmos, 2008, Robinson et at., 2008, Giurazza et al., 2012, Hishmat et al., 2014, Karell et al., 2016, and Mahfouz et al., 2016) (See Table 1 for list of the regions evaluated). Once the bones had been exported and measurements taken, regression equations developed for use on dry bones by Byrd and Adams (2003) were tested to assess their efficacy in relation to identifying the likelihood that bones originated from the same individual as they would in a dry bone commingling scenario.

Methods: Segmentation and extrapolation of the scans

For the extrapolation of the 3-Dimensional models, each scan was segmented and rendered in the workstation before being uploaded in the software for the application of the sorting techniques (Imaging, 2009, Kranioti and Senck, 2012, and FEI, 2015).
In AMIRA(V.5.3.0). It was possible to obtain a 2-Dimensional image through displaying the orthoslices (xy, xz and yz), selecting the desired density of the elements to display

(230.064 in this case) and adding the voltex needed for the segmentation step (See figure 1) (Kranioti and Senck, 2012). The semi-automated procedure described by Kranioti and Senck (2012) was utilised to isolate and extrapolate the single bones. In each case since the software was unable to extend the selection parameters identified for one slice to the whole bone, the whole region of interest was selected with the 'Magic wand' tool (See Figure 2). Before the final validation it was therefore necessary to adjust the threshold to obtain only the cortical bone of each of the elements.
With this procedure, there was also the possibility of selecting and deselecting the pixels to add or eliminate them from the area to be rendered (Kranioti and Senck, 2012). Before the exportation of the bones in the measuring software, visualisation of the new surface created with the previous segmentation was undertaken through the option 'Surface Generation' with the application of the command 'Material Statistic'.

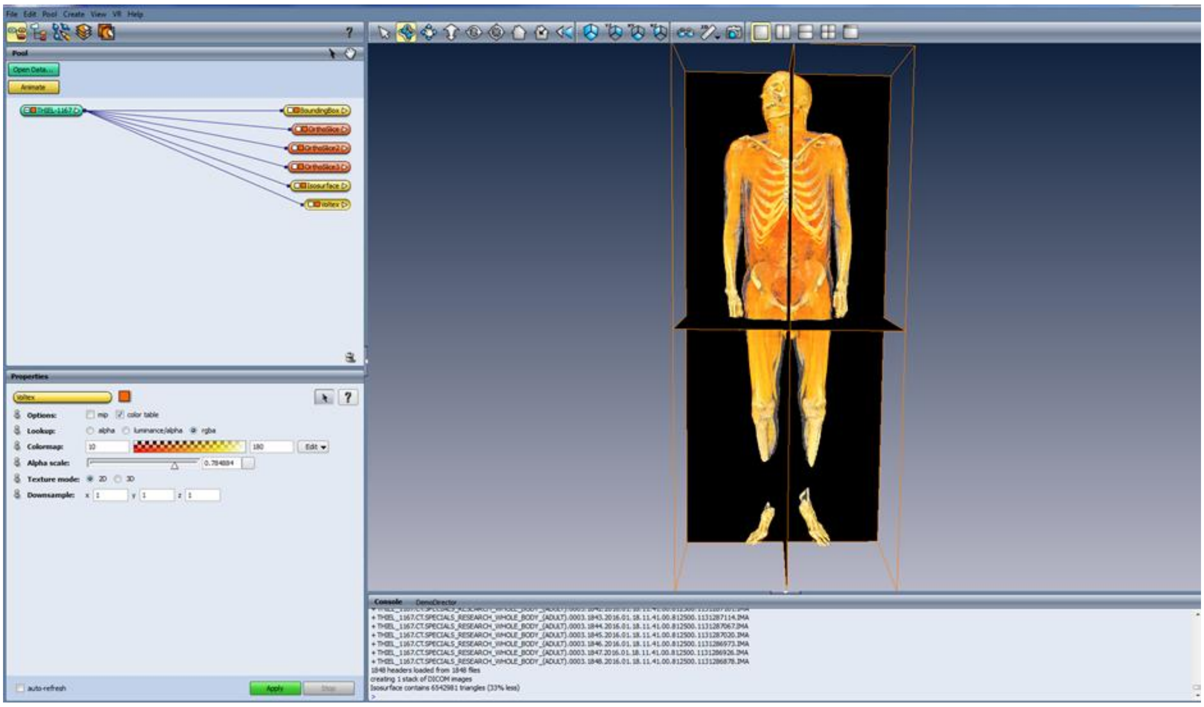

Figure 1 Displaying of the Voltex in Amira

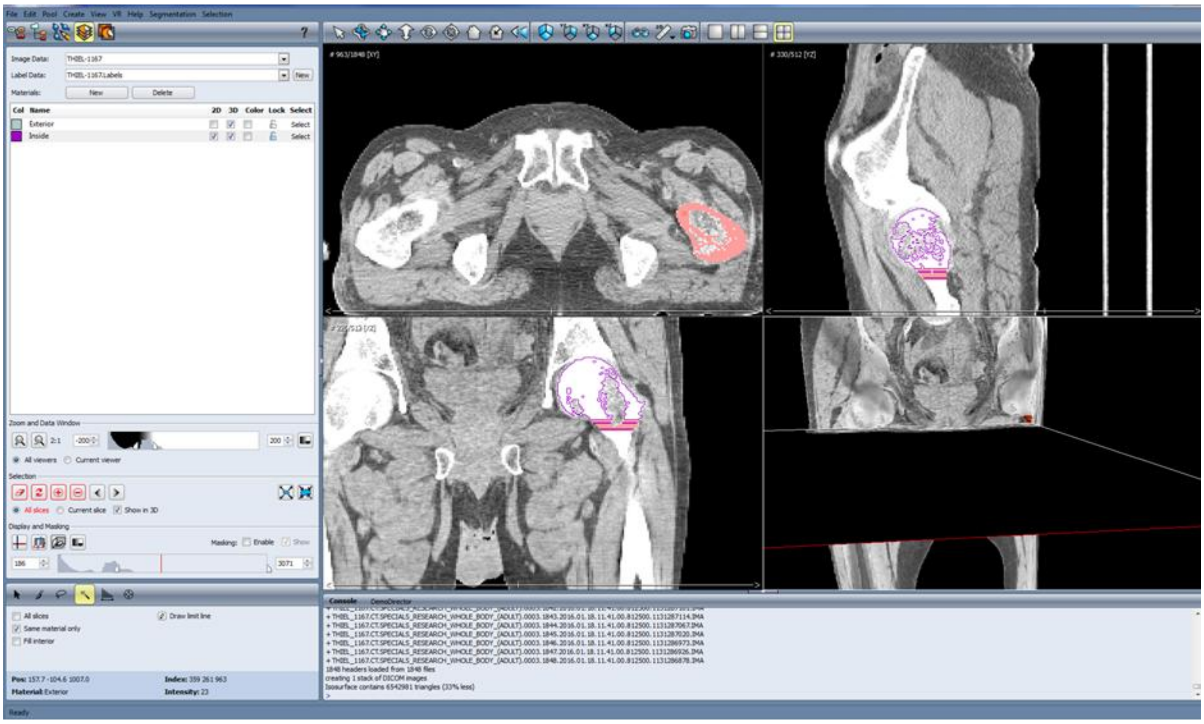

Figure 2 "Magic Wand" tool for the selection of the area of interest

Methods: Linear measurements

Taking into consideration the existing literature in the field, both past and present and based upon a comparison between left and right sides, a dataset of 29 linear measurements was created (Adams and Byrd, 2002, Bidmos, 2008, Buikstra and Ubelaker, 1994, Byrd and Adams, 2003, Chibba and Bidmos, 2007, Dedouit et al., 2007b, Giurazza et al., 2013, Guerrero Rodríguez et al., 2016, Moore-Jansen et al., 1994, Simmons et al., 1990, Stull et al., 2014).

The parameters included in the measurements are: total length of the bony element, diameter, distances between regions of the left and right sides of: Humerus, ulna, radius, femur, tibia and fibula (See the complete list of measurements with definition and description in Table 1).

Table 1 Measurements list and definitions

Post-cranial skeletal element	Measurement	Definition and description of the virtual tool procedure
Humerus	MXLH (Maximum Length) APHD (A-P diameter of the	Measure from the highest point of the head to the lowest in the trochlea; From the anterior and posterior surfaces of the

	head)	head;
	VHD (Vertical diameter of the head)	Measured from the highest to the lowest points at the articular surface of the humeral head;
	MXDM (Maximum diameter of the midshaft)	Taken M-L below the deltoid tuberosity;
	CTB (Capitulum Trochlea Breadth)	Width between the Capitulum and Trochlea in the distal epiphysis;
	EBH (Epicondylar Breadth)	From the lateral epicondyle, the most projected point, to its correspondent in the medial condyle.
Radius	MXLH (Maximum length)	Measure starting from the head of the radius, the most proximal point, to the lowest point in the styloid process;
	SDMS (Sagittal diameter at the midshaft)	Measured from the anterior to the posterior surfaces at the midshaft;
	TDMS (Transverse diameter at the midshaft)	Measured from the medial to the later surfaces at the midshaft;
	MXDRH (Maximum diameter of the radial head)	Measure the maximum distance around the radial head;
	MDRT (Maximum diameter on the radial tuberosity)	Measure the maximum distance around the tuberosity's shaft.

Ulna	PLH (Physiological length) MXLH (Maximum length) SBH (Semilunar Breadth) MND (Minimum Diameter) DVD (Dorso-volar A-P diameter) MLTD (M-L Transverse diameter)	Measure from the coronoid process, the deepest point, to the distal head of the ulna, at the lowest point; Measure from the highest point in the Olecranon, to the lowest in the styloid process; From the midpoint of the radial notch, at the edge in the middle of the semilunear notch; Measure the diameter in proximity of the distal end in the least area; At the maximum development of the crest in the diaphysis, between the A-P surfaces; At the maximum development of the crest in the diaphysis, between the M-L surfaces;
Femur	MXLH (Maximum length) MXHD (Maximum head diameter) EBH (Epicondylar breadth) APSD (A-P subtrochanteric diameter)	Measure from the highest point in the femoral head, to the lowest point on the distal surface of the inferior condyles; Measure, along the edges of the auricular surface, the maximum diameter; Measure from the most protruding point on the medial and lateral epicondyle; Under the lesser trochanter, measure between the A-P surfaces;

	MSD (Midshaft A-P diameter) UBH (Upper Breadth)	Measure at the midshaft of the diaphysis between the A-P surfaces, in the most protruding area of the linea aspera; The two most protruding projection in the proximal epiphysis of the femur.
Tibia	LH (Length) MXPEBH (Maximum proximal epiphyseal breath) MXDNF (Maximum diameter at the nutrient foramen) TDNF (Transverse diameter at the nutrient foramen)	Measure from the highest point in the articular surface of the proximal diaphysis, from the lowest point in the medial malleolus; Measure on the proximal epiphysis, the most protruding points on the medial and lateral sides; At the same height of the nutrient foramen, between the posterior surface and the anterior crest; Diameter taken perpendicular to the one described above.
Fibula	MXDMS (Maximum diameter at the midshaft) MXLH (Maximum length)	Measure in the midshaft the maximum diameter; From the highest point in the head to the lowest point in the malleolus;
Key terms for the description: A-P= Antero-Posterior measurements; M-L= Medio-Lateral measurements.		

Methods: Measuring process

In order to apply the selected measurements to the 3-Dimensional bones, created using AMIRA (V.5.3.0), the examiner imported the STL files on the software MeshLab (v.2016.12), an open source program used for computation of 3D models. The procedure was conducted through the application of the tool 'Measures'. Once this had been done it was possible to undertake the measurements from the 3D model using the measuring tool provided within the software and the landmarks identified on the bones (See Figure 3). In order to examine the repeatability of the measurements, intra and inter observer tests were run.

The intra observer test was conducted by the researcher in a different session from the first and the inter observer analysis involved a second examiner with the same level of experience, who was provided with instructions on the use of the software and a table of the measurements and landmarks required. All the data obtained by the three sessions (first, intra and inter) were analysed with the program SPSS Statistic, using a Two-Way Mix model with an absolute agreement and 95% confidence (Guerrero Rodríguez et al., 2016).

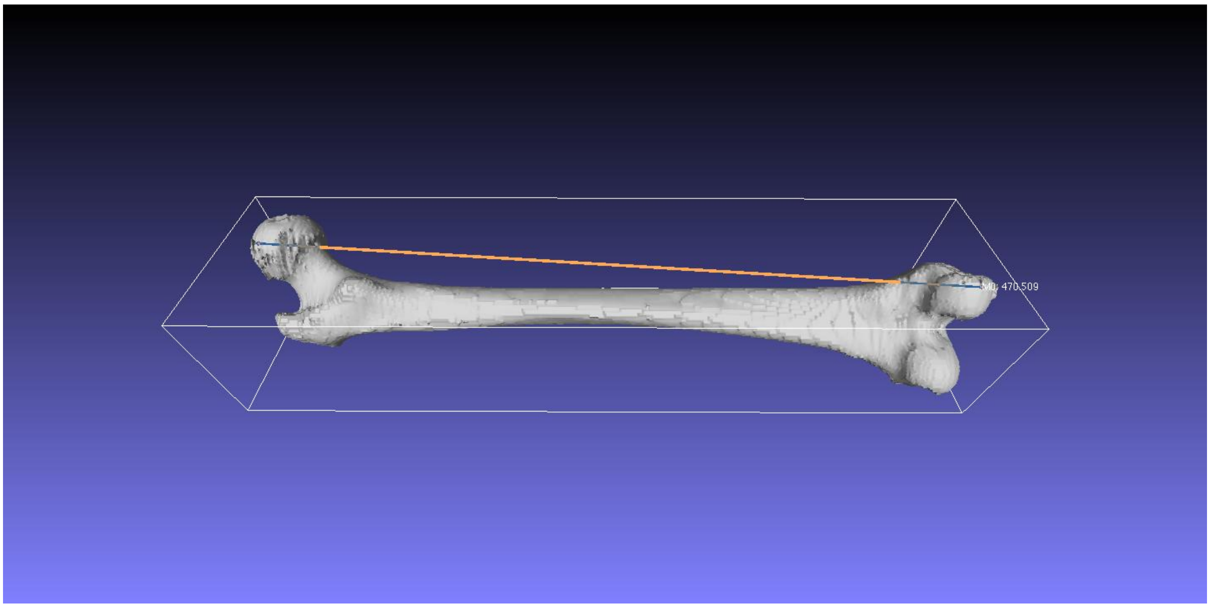

Figure 3 MXLH, Maximum length, of left femur measured in MeshLab.

Methods: Application of osteometric sorting models for linear measurements

For the sorting procedure, the examiner applied two sets of regression models, developed by Byrd and Adams (2003). The first set (test one) was originally developed by the authors from research in which all of the bony elements had a high level of preservation. The second test involved a higher degree of fragmentation of the measured bones and therefore there were less measurements included in the equation (Byrd and Adams, 2003). (See Table 2 for the complete list of regression equations).

Both models provide a predicted value and the results of the regression of one bone (dependent variable) from a second one (independent variable), to compare against a true value, a natural logarithm of the measurements' sum of the regressed bone. The hypothesis of two elements belonging to the same individual is accepted in the case in which the true value falls into the confidence interval applied to the predicted one (Byrd and Adams, 2003).

The first step includes the sum of all the measurements of each element and the conversion of each sum in a natural logarithm, in order to not compute numbers that are too elevated (Byrd and Adams, 2003). As a result, a standard deviation (SD), mean and standard error of the mean (SE) was established for each variable and the confidence intervals were calculated through the formula provided by Gules and Klepinger (1988). After those calculations the examiner possesses the variables to process the two sets of regression models.

Table 2 Regression models from Test 1 and 2, from Byrd and Adams (2003)

Regression models: Test 1	Regression models: Test 2
TIB=1.08(FEM)-0.78	HUM=1.08(RAD)-1.27
TIB= 0.65(ULN)+3.60	HUM=1.04(ULN)-1.47
TIB= 0.96(RAD)+0.77	HUM=1.18(FEM)-2.98
TIB=1.09(HUM)-0.54	HUM=1.02(TIB)+1.97
FEM=0.59(ULN)+4.08	RAD=0.84(ULN)+0.34
FEM=0.84(RAD)+1.74	RAD=0.96(FEM)-0.96
FEM=1.0(HUM)+0.28	RAD=0.81(TIB)-0.02
ULN=1.03(RAD)-1.78	ULN=1.02(FEM)-0.87
ULN=1.23(HUM)-3.58	ULN=0.85(TIB)+0.11
RAD=1.04(HUM)-0.81	FEM=0.74(TIB)+1.45

The results:

Inter and Intra observer error for osteological linear measurements

An Intra Class Correlation Coefficient Analysis was run on the sets of measurements taken by the first and second examiner. A high reliability has been found in both the intra-observer, with a value of 0.990, and in the inter-observer analyses, with 0.981 (See Table 3 and Table 4), so an overall elevated agreement has been established (Guerrerro Rodríguez et al., 2016).

Table 3 Intraclass correlation for the intra-observer reliability

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.980[a]	.975	.984	98.325	323	323	.000
Average Measures	.990[c]	.987	.992	98.325	323	323	.000

Table 4 Intraclass correlation for the Inter-observer reliability

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.962[a]	.953	.970	51.832	312	312	.000
Average Measures	.981[c]	.976	.985	51.832	312	312	.000

Regression models for osteometric sorting

The results obtained from the two models of regression equations were computed for the purpose of statistical analysis. Therefore, the examiner obtained four different scenarios: firstly there was either an acceptance or rejection of the null hypothesis of having two elements belonging to the same individual, this in turn had the potential to give Type 1 and 2 errors. Type 1 error determines a rejection of the null-hypothesis when the elements are actually belonging to the same individual. On the other hand, in

Type 2 errors there is an acceptance of elements originating from different individuals when in reality, they do not. The examiner computed 1908 comparisons for the first set of regression models listed in Test one, obtaining: 292 true acceptances were possible but only 75 were achieved by the software and on 1616 possible true rejection only 1127 were achieved. For the second set of regression equations the total number of comparison is 1326, in which on the 210 possible acceptances only 49 were achieved; while on the 1116 possible rejection only 917 were found by the software. Therefore, for Test one, 25.6 % (75 on 292 possible) of true acceptances and 69.7 % of true rejections (1127 on 1616 possible) has been achieved. Regarding Test two 23.1% of true acceptances had been achieved (49 on the 210 possible) and 82.1% of true rejections (917 on the 1116 possible).

Table 5 Results for the regression models from the first test.

Tot. comparisons	**True acceptance**	**True rejection**
Tibia-Femur (144)	62.5%	55%
Tibia-Ulna (120)	0%	100%
Tibia-Ulna (120) **No ulna length**	0%	100%
Tibia-Radius (120)	0%	95%
Tibia-Humerus (172)	83.3%	13.5%
Femur-Ulna (140)	0%	85%
Femur-Ulna (140) **No ulna length**	95.2%	11.9%
Femur-Humerus (192)	83.3%	11.9%
Femur-Radius (140)	0%	85%
Ulna-Radius (100)	0%	100%
Ulna-Radius (100) **No ulna length**	0%	100%

Ulna-Humerus (140)	0%	100%
Ulna-Humerus (140) **No ulna length**	0%	100%
Radius-Humerus (140)	100%	16.6%

Table 6 Result for the regression models from the second test.

	True Acceptance	**True Rejection**
Humerus-Radius (140)	0%	100%
Humerus- Ulna (140)	0%	100%
Humerus- Femur (154)	0%	100%
Humerus -Tibia (168)	0%	100%
Radius -Ulna (100)	100%	0%
Radius -Femur (120)	0%	100%
Radius -Tibia (120)	25%	72%
Ulna- Femur (120)	0%	100%
Ulna-Tibia (120)	0%	100%
Tibia-Femur (144)	100%	24.1%

Discussion:

In light of the increasing application of CT scan in the forensic anthropological field this study sought to test whether models designed for sorting commingled scenarios, originally developed for dry bones, could have the same applicability for virtual models, in terms of validity and reliability of the results.

Osteometric sorting models rely on a set of regression equations which utilise data taken through physical measurements of dry elements. Being aware of the extent to which this technique may be applied in a novel way, it is essential to test these methods to produce results which are able to stand in a courtroom against the standards of validity and reliability that the legislation in the field requires (National Research Council 2009, The Law Commission, 2011, and Executive Office of the President, 2016). Taking into consideration the ethical issues of exposing living subjects to radiations or the intentional disruption of human remains, this project used already existent cadaveric scans, with the approval of the University of Dundee. Therefore, an evaluation of how the study might be affected with a sample originally scanned for different research questions is also brought to light in the project.

Osteometric sorting models for linear measurements

After an evaluation of the results from both the regression equations within test 1 and the regression equations within test 2 there were a noticeably low percentage of true acceptance of the null-hypothesis (25.6% for Test 1 and 23.1% for Test 2), compared with an elevated number of true rejections, for both tests. This has important implications for the segregation process. It is important to understand that when resolving commingled remains, the possibility of having two elements generated from the same individual is not a sufficient proof of association but being able to exclude the pairing of two elements is as important, if not more so, than a positive match (Byrd and Adams, 2003 and Byrd, 2008). In fact, the authors advise that this sorting technique is always used with other segregation methods for the reliability of the results (Byrd and Adams, 2003 and Byrd, 2008).

Moreover, in the first set of regression models, the authors noted more variability in the percentages of acceptance, rejections and errors (See Table 5 for details). Besides, in the second set of regression models, there was an overall overestimation or underestimation of the predicted values (e.g. the radii tend to be overestimated when regressed on ulnae and the humeri underestimated when regressed on the radii). This reduced the variability of the percentages (e.g. acceptance, rejections and errors) (See Table 6 for details).

There are a number of factors that could have affected the reliability of these results. Firstly, the decision to create a new dataset of selected measurements from those tested by Adams and Byrd (2003), in order to include a wider range of studies on linear osteometric measurements and commingled scenarios could have had an impact on the results (Simmons et al., 1990, Buikstra and Ubelaker, 1994, Moor-Jansen et al., 1994, Adams and Byrd, 2002, Byrd and Adams, 2003, Chibba and Bidmos, 2007, Dedouit et al., 2007, Bidmos, 2008, Giurazza et al., 2013, Stull et al., 2014, and Guerrero-Rodrìguez et al., 2016).

Another important factor is represented by the quality of the representation of the surface of the bones in the 3D model. Adams and Byrd (2003) point out the invalidity of the statistical models where the surface of the bone is disrupted causing inaccuracies in measurements. The surfaces of the bones in the 3D models were unclear in places due to the elevated pixilation of the segmented elements, causing issues with location of bony landmarks and therefore potential issues with measurement accuracy. There were also issues in relation to t the application of the methodologies which originated from the paper of Byrd and Adams (2003). Some of the passages in the article which described the method of computing the equations (e.g. from the mathematical formula to obtain the confidence intervals to how to apply the predicted values) are presented in, and originate from, a different study by Giles and Klepinger (1988). Moreover, not all the variables are explained in depth, such as the method for calculating the SE (Standard Error) in the formula. This creates a lack of basic information, especially for users without a deep knowledge of statistical computations and as a result it is recommended that these analyses are used with caution.

Conclusion

This study tested the applicability of linear measurements and regression equations originally developed for sorting dry specimens on 3-Dimensional models virtually reconstructed from whole-body CT scans of a cadaveric population.

The results suggest that use of these methods can't be reliably applied to bones extracted from CT scans (Byrd and Adams, 2003)

Among the limitations highlighted in the study, the quality of the bones' surface during the rendering procedure, appears to reduce considerably the efficacy of the models and issue which has been highlighted in the existing literature (Byrd and Adams, 2003, and Byrd 2008). This can be influenced by the thickness of the slices tested and further research is needed to fully explore the effects of those factors.

Moreover, the lack of clear statistical procedures when computing the equations could cause misinterpretation in the application of the procedures and compromise the reliability of the results.

Therefore, the examiners recommend further work seeking for more accurate procedural description, a larger study sample and considerations regarding the thickness of CT scans tested.

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